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CTL epitopes of FMDV determined by the NetMHCpan-driven predictions of SLA/peptide binding, confirmed by tetramer complex formation and staining

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Abstract

To date little has been reported about porcine T cell epitopes during viral disease. In this study, we describe the application of a state-of-the-art MHC/peptide predictor algorithm developed for Human Leukocyte Antigen (HLA) Class I genes, NetMHCpan. To analyze Swine Leukocyte Antigen (SLA) molecules SLA-1*0401 and SLA-2*0401, we used closely related HLA Class I molecules in the database to identify potential peptide epitopes from the P1/capsid protein region of foot-and-mouth disease virus (FMDV) strain A24. NetMHCpan predicted nine possible 9mer and 10mer peptide sequences that would bind SLA-1*0401 and ten for SLA-2*0401, with nine of ten predicted to bind both molecules. In a peptide dependent assay for MHC-peptide complex formation with recombinant SLA proteins, five of the predicted peptides bound SLA-1*0401 at significant binding affinities and four peptides bound SLA-2*0401. Having identified these FMDV peptides with potential for being epitopes for T cells restricted to these two SLA molecules, we prepared tetramers of MHC-peptide complexes as previously described for humans. Swine, homozygous for SLA-1*0401 and SLA-2*0401, were vaccinated with a viral vectored FMDV P1-capsid vaccine and boosted a month later. Analysis of CD8+ cytotoxic T lymphocytes (CTLs) from these animals showed FMDV specific killing of virus infected PK-15 target cells, which are also matched at SLA. One of the five tetramers generated for SLA-1 specifically stained CD8+ T cells from these vaccinated animals. The four other SLA-1 tetramers and the four generated for SLA-2 showed marginal reactivity. These data indicate that we have identified a porcine FMDV specific T cell epitope that may be immunodominant for animals of the SLA-*0401 genotype.

Introduction

Major Histocompatibility Complex class I (MHC I) molecules are highly polymorphic proteins that bind peptides derived from the cellular protein metabolism with a length of 8-11 amino acids. They are found in all vertebrates and play a crucial role in the cellular immune response against viruses and other intracellular pathogens. Surface exposed MHC/peptide complexes serve as targets for peptide-specific, MHC I restricted cytotoxic T cells. Endogenous peptides, therefore, are constantly presented on the cell surface signaling the current state of the individual cell to the immune system of the host. Specific T cells may become activated and kill the cell if the peptide presented is of foreign origin.

Both SLA-1*0401 and SLA-2*0401 are commonly expressed alleles in commercially available pigs. Here we have used the NetMHCPan predictor to predict peptide binders for these two molecules and further tested the peptides *in vitro* using a biochemical binding affinity assay (ELISA). Identified peptide binders were used to generate tetramers that were further tested for their ability to stain FMDV reactive T cells derived from pigs previously vaccinated with a viral vectored FMDV P1-capsid vaccine and boosted a month later.

Approach

(i) Select peptide sequences of interest within the FMDV A24 structural protein (P1) region using known binding motifs for the SLA-1*0401 allele

(ii) Run chosen peptide sequences through the NetMHCpan predictor algorithm to identify the peptides which are predicted to bind

(iii) Perform ELISA based testing of the predicted peptide binders to identify true binders from non-binders

(iv) Produce SLA-tetramers using FMDV derived SLA-1*0401 peptide binders

(v) Stain FMD reactive T cells from previously vaccinated pigs using SLA/FMDV-peptide tetramers

The A24 FMDV P1 region

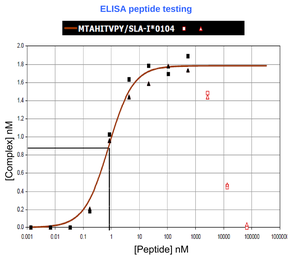
GAGQSSPATGSQNQSGNTGSIINNYYMQQYQNSMDTQLGDNAISGGSNE
GSTDTTSTHTTNTQNNDWFSKLASSAFTGLFGALLADKKTEETTLLEDRI
LTTRNGHTTSTTQSSVGVTHGYSTEEDHVAGPNTSGLETRVVQAERFYKK
YLFDWTTDKAFGHLEKLELPSDHHGVFGHLVDSYAYMRNGWDVEVSAVGN
QPNGGCLLVAMVPEWKEFDTREKYQLTLFPHQFISPRTNLTAHITVPYLG
VNRYDQYKKHKPWTLVVMVVSPLTVNNTSAAQIKVYANIAPTYVHVAGEL
PSKEGIFPVACADGYGGLVTTDPKTADPAYGKVYNPPRTNYPGRFTNLLD
VAEACPTFLCFDDGKPYVTTRTDDTRLLAKFDLSLAAKHMSNTYLSGIAQ
YYTQYSGTINLHFMFTGSTDSKARYMVAYIPPGVETPPDTPERAAHCIHA
EWDTGLNSKFTFSIPYVSAADYAYTASDTAETINVQGWVCVQITHGKAE
NDTLVVSVSAGKDFELRLPIDPRQQTTATGESADPVTTTVENYGGETQIQ
RRHHTDIGFIMDRFVKQDLSPTHVDLMQTHQHGLVGALLMATYYFSD
LEIVVRHEGNLTWVPNGAPESALLNTSNPTAYNKAPFTRLALPYTAPHRV
LATVYNGTSKYAVGGSGRRGDMGSLAARVVKQLPASFNYGAIKADAIHEL
LVRMKRAELYCPRPLLAIEVSSQDRHKQKIAPAKQ

NetMHCpan predictions and actual ELISA peptide binding

Allele	Peptide	Predicted Rank	Actual K$_D$ (nM)
SLA-1*0401	MTAHITVPY	0.01/SB*	0.80/SB
SLA-1*0401	SSVGVTHGY	0.15/WB*	433.00/WB
SLA-1*0401	AAKHMSNTY	1.00/WB	20000/NB*
SLA-1*0401	TVYNGTSKY	0.15/WB	87.00/SB
SLA-2*0401	MTAHITVPY	0.05/SB	7.00/SB
SLA-2*0401	SSVGVTHGY	0.60/WB	14.00/SB
SLA-2*0401	AAKHMSNTY	0.60/WB	20000/NB
SLA-2*0401	TVYNGTSKY	0.05/SB	20000/NB

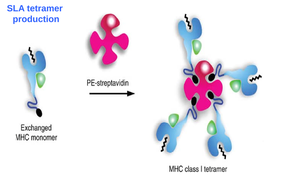
NetMHCpan version 2.2
Peptide length: 9
SLA-1*0401: Estimated prediction accuracy: 0.444 (using nearest neighbor HLA-B*1517)
SLA-2*0401: Estimated prediction accuracy: 0.393 (using nearest neighbor H2-Kk)
Rank Threshold for Strong Binding peptides (*SB): 0.100.
Rank Threshold for Weak Binding peptides (*WB): 1.000.
Non-binders (*NB) were assigned a K$_D$ of 20000 nM.

ELISA peptide testing



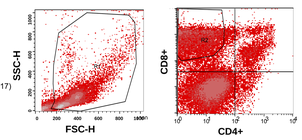
Selected peptides were tested and their K$_D$ binding values determined using a streptavidin/biotin interaction based ELISA catching correctly folded SLA complexes via the β$_2$m, using anti-β2m monoclonal antibody.

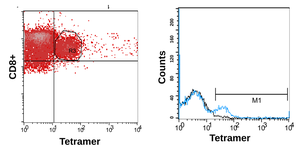
SLA tetramer production



Tetramer production figure taken from: Rodenko, B., M. Toebes, S. Reker Hadrup, W.J.E. van Esch, A. M. Molenaar, T. N. M. Schumacher & H. Ovaa. (2006) Generation of MHC-I complexes through UV mediated ligand exchange. Nature Protocols 1, 1120-1132.

Tetramer staining of FMDV reactive T cells





Specific T cells stained by the FMDV-peptide tetramer (blue) versus staining of the same T cell population by a negative control peptide tetramer (from Ebola virus, black). FITC labeled anti-CD4 (clone 74-12-4) and PE labeled anti-CD8 (clone 76-2-11) were obtained from BD (California, USA).

Conclusion

NetMHCpan predicted nine possible 9mer and 10mer peptide sequences that would bind SLA-1*0401 and ten for SLA-2*0401, with nine of ten predicted to bind both molecules. Five of the predicted peptides bound SLA-1*0401 at significant binding affinities and four peptides bound SLA-2*0401. One of the five tetramers generated for SLA-1 specifically stained CD8+ T cells from these vaccinated animals. The four other SLA-1 tetramers and the four generated for SLA-2 showed marginal reactivity. These data indicate that we have identified a porcine FMDV specific T cell epitope that may be immunodominant for animals of the SLA-*0401 genotype.